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Daleen Badenhorst, Vincent Herbreteau, Yannick Chaval, Marie Pages, Terence J. Robinson, et al.. New karyotypic data for Asian rodents (Rodentia, Muridae) with the first report of B-chromosomes in the genus *Mus*. *Journal of Zoology*, 2009, 279 (1), pp.44-56. 10.1111/j.1469-7998.2009.00588.x . hal-00545596

HAL Id: hal-00545596

<https://hal.science/hal-00545596>

Submitted on 15 May 2020

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New karyotypic data for Asian rodents (Rodentia, Muridae)

with the first report of B-chromosomes in the genus *Mus*

Daleen Badenhorst¹, Vincent Herbreteau², Yannick Chaval³, Marie Pages³,
Terence J. Robinson¹, Worawut Rerkamnuaychoke⁴, Serge Morand⁵, Jean-Pierre
Hugot⁶ & Gauthier Dobigny^{3*}

¹ Evolutionary Genomics Group, University of Stellenbosch, Botany and Zoology

Department, Private Bag X1, 7604 Matieland, South Africa.

² Territoires, Environnement, Télédétection et Information Spatiale (UMR TETIS

Cemagref-CIRAD-AgroParisTech) Maison de la Télédétection, 500 rue JF Breton, 34093

Montpellier Cedex 5, France.

³ Centre de Biologie pour la Gestion des Populations (UMR 022 INRA-IRD-CIRAD-

SupAgro), Campus de Baillarguet, CS30016, 34988 Montferrier-sur-Lez, France.

⁴ College of Veterinary Technology, Kasetsart University, 50 Paholyothin Rd, 10900

Bangkok, Thailand.

⁵ Institut des Sciences de l'Evolution (UMR 5554 CNRS-IRD-UM2), Université de

Montpellier II, Bâtiment 22, Place E. Bataillon, 34095 Montpellier, France.

⁶ Laboratoire Origine, Structure et Evolution de la Biodiversité (UMR 5202 CNRS-MNHN),

Muséum National d'Histoire Naturelle, 55 rue Buffon, 75005 Paris, France

* Corresponding author: Gauthier.Dobigny@ird.fr

Keywords: cytotaxonomy – systematics – sibling species – Rattini

Running title: Cytotaxonomy in Asian Rodents

Abstract (216 words)

Karyotypes of 18 rodent species collected in various localities in Thailand were analysed as part of an epidemiological survey of the region using conventional cytogenetic techniques. The aim was to re-assess the reliability of karyotype-based diagnoses of Thai rodents using an updated taxonomic framework. Species examined include *Menetes berdmorei* (Sciuridae), *Mus caroli*, *M. cervicolor* and *M. cookii*, *Hapalomys delacouri*, *Chiropodomys gliroides*, as well as several representatives of most of the lineages of the Rattini tribe i.e. *Rattus exulans*, *R. losea*, *R. tanezumi*, *Leopoldamys edwardsi*, *L. neilli*, *Maxomys surifer*, *Niviventer fulvescens*, *Berylmys berdmorei*, *B. bowersi*, *Bandicota indica* and *B. savilei* (Muridae). The first descriptions of G- and/or C-banding karyotypes are provided for several of these i.e., *B. savilei*, *L. edwardsi*, *M. surifer*, *B. berdmorei*, *B. bowersi*, *N. fulvescens* and *H. delacouri*. Although largely in agreement with available data, our findings on chromosomes morphology differ slightly from those published for *L. edwardsi*, *M. surifer*, *B. savilei*, and the two *Berylmys* species, *B. berdmorei*, and *B. bowersi*. In addition, we document the novel finding of B-chromosomes in the genera *Berylmys*, *Bandicota* and the emblematic *Mus*. Importantly, few species-specific chromosomal characters could be identified within most of the genera investigated in our study and, contrary to previous claims, the usefulness of karyotypes for diagnosing these Asian murid species appears limited.

INTRODUCTION

The non-ambiguous identification of species is critical for fundamental as well as applied research, as it is the departure point for many aspects of comparative biology including systematics (e.g., Sites & Marshall, 2003, and references therein), biodiversity assessments, conservation (Crandall *et al.*, 2000; Moritz, 2000), biogeography (e.g., Avise 2009), community ecology and pests control (e.g., Hoffman, 2002). In particular, the lack of accurate species-specific identification of hosts is an important consideration for current epidemiological surveys since 1) numerous pathogens are carried by a restricted range of hosts, and 2) each rodent species possesses its own life traits. These two aspects are pivotal since they may lead to important differences in the role a host species can play in circulation and transmission of pathogens. For instance, a species may be an important reservoir for a human pathogen while its close, and sometimes morphologically similar relatives, are not (e.g., the two cryptic species *Mastomys natalensis* and *M. erythroleucus*, and their association with the Lassa Fever arenavirus in Africa; Lecompte *et al.*, 2006). Additionally, some species-specific ecological characteristics (such as commensality and hence the propensity to transport with humans) and evolutionary properties (population structure and connectivity) are also central to epidemiological processes (reviewed in Mills & Childs, 1998). In such a context, karyotypic data has been shown to be valuable, especially for identifying rodents that belong to sibling species complexes.

We carried out a survey of various rodent species found in different localities in Thailand as part of an epidemiological survey in this region. In particular, we focused on murids (rats and mice) that are suspected of being important reservoirs for several human pathogens (i.e., those responsible for hantavirus-mediated hemorrhagic fevers, typhus, leptospirosis, among others).

The first goal was to develop identification “tools” for use at the species-level. That this approach has application is illustrated by the Asian Hantaviruses (responsible for lethal haemorrhagic fevers) / rodents association found in Cambodian *Rattus* specimens that could not be discriminated at the species level (Reynes *et al.*, 2003).

Asia is the area of origin for the *Rattus sensu lato* complex (i.e., *Rattus sensu stricto* and allied genera), a complex that includes the biological important index species, *R. norvegicus* (Aplin *et al.*, 2003). Surprisingly, given their epidemiological importance, karyotypic data on species in this complex are limited. Even more importantly, numerous systematic modifications (reviewed in Wilson & Reeder, 2005) have been proposed since the pioneering cytotaxonomic work published in the late 1960s to the 1970s (see Marshall, 1977, and references therein), further complicating interpretations of correspondence between karyotype and host species. For example, many (if not most) of the taxa that were originally considered as different *R. rattus* subspecies (e.g., *tanezumi*, *argentiventer*, *rattus*) are now regarded as synonyms or have been elevated to distinct species. In the same manner, many congeneric species have been split into different genera (e.g., *Berylmys*, *Maxomys*, *Leopoldamys*, *Niviventer*). Consequently, species-specific karyotypes are often difficult to delimit, thus making a cytotaxonomic approach to species identification problematic.

In an attempt at redress, we performed a conventional cytogenetic analysis of 18 rodent species: *Menetes berdmorei* (Sciuridae), *Mus caroli*, *M. cervicolor* and *M. cookii*, *Chiropodomys gliroides*, *Hapalomys delacouri*, as well as representatives of most of the lineages within the Rattini tribe (*sensu* Lecompte *et al.*, 2008), i.e. *Rattus exulans*, *R. losea*, *R. tanezumi*, *Leopoldamys edwardsi*, *L. neilli*, *Maxomys surifer*, *Niviventer* cf. *langbianis*,

Berylmys berdmorei, *B. bowersi*, *Bandicota indica*, *B. savilei*. Our aim was to re-assess the reliability of karyotype-based diagnoses of Thai rodents, in an updated taxonomic framework.

MATERIAL and METHODS

Thousands of rodents have been trapped and are currently under investigation as part of a long-term epidemiological survey of rodent-borne pathogens in South Eastern Asia. Among these, sixty five specimens were collected in 2006 and 2007 in Northern and North-Eastern Thailand in the vicinity of the settlements Loei (17°29N 101°43E), Phrae (18°09N 100°08E) and Kalasin (16°26N 103°31E) using locally made locally made cage-traps. Except where otherwise detailed, taxonomy follows Carleton & Musser (2005). Species identifications were based on external measurements, morphological features, and, in females, mammae formula (Marshall, 1977; Corbet & Hill, 1992; Aplin *et al.*, 2003). Moreover, taking into account the ambiguous status of some of these diagnostic characters, most of the murine specimens were also investigated using molecular analyses (Tab. 1) involving several mitochondrial (cytochrome b, CO1, and 16S rDNA) and nuclear (IRBP) gene fragments. The DNA-based analyses will be presented elsewhere as a separate phylogenetic study (Pagès *et al.*, in prep.). Importantly, however, and pivotal to the present work, our species-specific identifications relied on correspondence in both morphological and molecular data.

The majority of chromosome preparations were done in the field using the air-drying method on femoral bone marrow following yeast stimulation (Evans *et al.*, 1963; Lee & Elder, 1980). In eleven instances (Tab. 1), intercostal tissue was sampled to establish fibroblast cell cultures using conventional techniques. Freshly prepared hypotonic (0.75 M KCl) and fixative (acetic acid-methanol 1:3) solutions were routinely used. Cell suspensions were stored in fixative and

transported to the laboratory where conventional staining, G- and C-banding (Seabright 1971; Sumner 1972) and image capture using the Genus software (Applied Imaging), were performed. A minimum of five complete metaphases were analysed for each specimen. Diploid (2n), fundamental (NF; i.e., total numbers of chromosomal arms in one cell) and autosomal fundamental (NFa; i.e., NF excluding sex chromosomes) numbers were determined, and karyotypes were arranged according to chromosomes morphology and size (conventional staining) and/or banding patterns. Chromosome nomenclature for *Rattus* followed Yosida (1976, 1977a).

RESULTS and DISCUSSION

All specimens were analyzed using conventional staining, thus allowing an overall description of the species-specific karyotypes (Tab. 1). In addition, G-banded karyotypes were constructed where feasible. However, in several instances, slight variation and/or ambiguities in NF/NFa values were detected through conventional staining (Giemsa only) reflecting the difficulty in categorizing acrocentric/subtelocentric chromosomes where chromosomal contraction can be a confounding factor. Although not uncommon in mammalian cytogenetics, subtle differences in chromosome morphology (such as telocentric vs. subtelocentric) has been considered important in murine cytotaxonomy where it has been used to underpin the species identification of several taxa (e.g., Marshall, 1977, p. 407-408). In an attempt to constrain ambiguities introduced by terminology, we have chosen to view all chromosomes that have no visible short arm as telocentric and those that possess minute arms as acrocentric elements (subtelocentric chromosomes in some schemes) for NF/NFa assessment. If the short arms were more pronounced and clearly and repeatedly observed, chromosomes were considered to be as bi-armed (submetacentric and metacentric).

144

145 *Menetes berdmorei* (Blyth, 1849) (Sciuridae)

146

147 The only squirrel (which is a sciurid, not a murid rodent) investigated in this study had a
 148 $2n=38$ chromosome number. Most of the chromosomes were metacentric or submetacentric
 149 except for the five smallest pairs that were either telocentric or acrocentric (NF=76, NFa=72).
 150 This is in agreement with data previously published by Viegas-Péquignot *et al.* (1986) and
 151 Richard *et al.* (2003) on this species, and also specimens analysed by Marshall (1977) from
 152 Thailand. As a result the karyotype is not repeated here.

153

154 *Rattus exulans* (Peale, 1848), *R. losea* (Swinhoe, 1871) and *R. tanezumi* (Temminck, 1844)

155

156 In total 26 specimens of *Rattus sensu stricto* were karyotyped (Tab. 1; data not shown). These
 157 included *R. losea* ($n=5$), *R. tanezumi* ($n=13$) and *R. exulans* ($n=8$). All but one specimen were
 158 determined to have a diploid number of 42 with NF values ranging from 56-61, 58-60 and 54-
 159 59 in *R. exulans*, *R. losea* and *R. tanezumi*, respectively. The $2n=43$ *R. tanezumi* specimen
 160 was characterized by the presence of a G-negative and C-positive B-chromosome (data not
 161 shown), as has previously been documented in this taxon (Yosida, 1976, 1977b; Gadi &
 162 Sharma, 1983). The largely overlapping NF values are attributable to variation in the short
 163 arms (p arms) of several pairs resulting in their classification as acrocentric and/or
 164 submetacentric homologs (see Table 2). The polymorphisms most likely correspond to
 165 variation in pairs 1, 9 and 13 of *Rattus r. tanezumi* (now named *R. tanezumi*; see Wilson &
 166 Reeder, 2005) *sensu* Yosida and colleagues (1971a, 1971b, 1976 and 1977a). This variation in
 167 centromere position has been noted in several *Rattus* species and subspecies (Yosida *et al.*,
 168 1971a, 1971b, 1974; Yosida, 1973, 1977a; Yosida & Sagai, 1973, 1975; Gadi & Sharma,

1983; Motokawa *et al.*, 2001) and is interpreted to result from pericentric inversion (Yosida *et al.*, 1971a, 1971b; Yosida, 1976, 1977a) and/or heterochromatin amplification/deletion (Yosida & Sagai, 1975; Baskevich & Kuznetsov, 1998). However, the chromosomes and/or the segments involved are small and thus difficult to interpret, even when using G-banding, and the mechanism(s) underpinning this variation remains moot.

Importantly, Marshall (1977) proposed a number of apparently fixed (diagnostic) features among *R. exulans*, *R. losea* and *R. tanezumi* (which is synonymous with *R. r. thai sensu* Marshall; see Wilson & Reeder, 2005) in Thailand. On the basis of conventional staining, Marshall argues (pp. 407-408) that: i) pair 1 is acrocentric in *R. tanezumi* and submetacentric in *R. exulans* and *R. losea*, ii) pair 9 is submetacentric in *R. losea* but acrocentric in *R. exulans* and *R. tanezumi*, and iii) pair 11 (most probably corresponding to pair 13 *sensu* Yosida *et al.*) is submetacentric in *R. exulans*, whereas it is acrocentric in *R. losea* and *R. tanezumi*. However, taking other studies into account (see above) and our own data (Table 2), it appears that none of these chromosomal morphs (1, 9 or 13) can be considered as species-specific. Moreover, heterozygotes for each of these three pairs were found in *R. exulans* and *R. tanezumi* (our study, Tab. 2), suggesting that these chromosomal polymorphisms were present in their last common ancestor and are therefore ancestral to the *Rattus sensu stricto* clade (Yosida *et al.*, 1971a, 1971b; Yosida & Sagai, 1975, Yosida, 1977) and date back ~2.2 My (Robins *et al.*, 2008).

Leopoldamys edwardsi (Thomas, 1882) and *L. neilli* (Marshall Jr, 1976)

The five *L. edwardsi* specimens investigated in this study all display identical karyotypes (Fig. 1) with 2n=42 chromosomes. Most of the autosomes are acrocentric, except for four of

the five largest autosomal pairs (pairs 1, 2, 3 and 5 in Fig. 1) which possess short arms, as well as the three smallest ones which are metacentric (pairs 18, 19 and 20). The X is a medium size acrocentric chromosome, whereas the Y is C-positive and dot-like (and most likely acrocentric) element (NFa=54). Clearly observable blocks of C-positive heterochromatin were detected in several pericentromeric and telomeric regions, as well as in an intercalary position on one of the largest pairs (Fig. 1b; pair 2 on Fig. 1a).

Although *L. edwardsi* (from an unknown locality) was recently proposed to have $2n=44$ chromosomes (Sablina *et al.*, unpubl., quoted in the “Atlas of Mammalian Chromosomes”, ed. S.J. O’Brien *et al.*, 2007), our karyotypic results are in agreement with other reports (Yong, 1970; Rickart & Musser, 1993, and references therein). However, there is also a difference in the morphology of the Y chromosomes: the Y identified by Yong (1970) is a normal sized acrocentric, while our Y clearly appears as a dot like element (Fig 1b), suggesting there may be more fundamental differences underlying these observations.

A single *Leopoldamys* female trapped in the vicinity of limestone karsts was referred to as *L. neilli* on the basis of morphology as well as unambiguous molecular results (Pagès *et al.*, in prep.). Even though our preparation was of poor quality (data not shown), making G-banding analysis problematic, the specimen unambiguously displayed $2n=44$ chromosomes, with two large submetacentric and two small metacentric pairs (NF=52). These findings are in good agreement with previous reports (NF=52 in Marshall, 1977) but differ from the NF=50 noted by Rickart & Musser (1983).

It would appear, therefore, that the two *Leopoldamys* species can be distinguished by karyotypes: *L. edwardsi* with $2n=42$ /NF=56 and *L. neilli* with $2n=44$ /NF=52. A third species,

L. sabanus, which is morphologically similar to *L. edwardsi*, has also been described from Thailand (Wilson & Reeder, 2005). The *L. sabanus* karyotype differs slightly from that of *L. edwardsi* since Marshall (1977) recorded a $2n=42/NF=54$ complement for this species. Unfortunately, no material was available to us to address the delimitation of the *L. edwardsi* / *L. sabanus* species. However, we anticipate that karyotypic differences between these two taxa are subtle, therefore unreliable for diagnostic purposes.

Niviventer fulvescens

Taxonomy within *Niviventer* is in need of revision (Wilson & Reeder, 2005). The results of our preliminary molecular (Pagès *et al.*, in prep.) and morphological (Chaval, unpubl.) investigations are concordant and show that the three specimens analysed karyotypically by us form a natural grouping and are referred to herein as *N. fulvescens* (Pagès *et al.*, in prep.). Of these three specimens, a male was used to establish a fibroblast culture but the resulting lines appear to have undergone *de novo* rearrangement in culture. Nonetheless, the rearranged karyotype corresponds relatively well to the bone marrow karyotypes of the other two specimens which were both characterized by $2n=46$ (NFa=52; Fig. 2). All chromosomes are acrocentric except for the submetacentric pair 2, as well as the three of the shortest autosomal pairs which are metacentric. The X is a large acrocentric chromosome, and the Y is a tiny acrocentric element which is entirely C-positive (data not shown). As far as we know, the G-banding karyotype of *N. fulvescens* (Fig. 2) is presented here for the first time.

There are few published karyotype reports on subspecies/species of *Niviventer* and these are limited to using conventional staining, the only exception being Wang *et al.* (2003) on *N. confucianus*. Except for tiny differences in centromeric placement, *N. rapit*, *N. bukit*, *N.*

hinpoon (Yong, 1969; Markvong *et al.*, 1973; Marshall, 1977), *N. langbianis* (Baskevich & Kuznetsov, 2000), *N. fulvescens* (Duncan *et al.*, 1970, 1974) and *N. cremoriventer* (Yong, 1969) are all invariant and closely resemble the cytotype that we present here for *N. fulvescens*. It is important to note that *N. rapit* and *N. bukit* from Thailand have since then been collapsed into *N. fulvescens* (see Musser, 1981; Abe, 1983). The cytogenetic criteria proposed to discriminate all these *Niviventer* species are based on subtle shape differences of some pairs (see Table 2 in Wang *et al.*, 2003) which are most probably due to variation in heterochromatin, as observed between *N. tenaster*, *N. fulvescens* and *N. langbianis* (all from Vietnam) following C-banding protocols (Baskevich & Kuznetsov, 2000). Although we cannot state with certainty, it is highly probable that karyotypic variations within the genus will be too obscure to provide reliable cytotaxonomic identification.

Maxomys surifer (Miller, 1900)

Five males and one female of *M. surifer* were analysed and the G-banded karyotype is presented for the first time (Fig. 3). All specimens displayed $2n=52$, with seven metacentric (pairs 4, 5 and 14 in Fig. 3) or submetacentric (pairs 10, 12, 18 and 24) chromosomal pairs (NF=66). The Y chromosome is the smallest element, is entirely heterochromatic (Fig. 3b), and appears acrocentric in morphology. This is in agreement with available data for *M. surifer* from South East Asia (see Rickart & Musser, 1993, and references therein) including Thailand (Marshall, 1977). However, some differences exist between these studies and the results of the present report especially with respect to the identification of the X chromosome. The X was described as a metacentric in specimens collected from localities in Thailand (Marshall, 1977), Vietnam and Malaysia (see Rickart & Musser, 1993, and references therein), resulting in $2n=52/NFa=62$. In sharp contrast, the X chromosome is one of the

largest chromosomal elements in specimens analysed by us, and is clearly acrocentric. Zoo-FISH analysis using a *R. norvegicus* X painting probe (Badenhorst *et al.*, unpubl.) allowed us to confirm this result. Our *M. surifer* specimens are consequently characterized by NFa=64, and not NFa=62. When one considers our findings on the morphology of the X chromosome, the difference in NFa (NFa=62 vs 64) may be due to (i) a misidentification of the X in the past, (ii) a polymorphism that would involve the X chromosome, or (iii) the co-existence of different but cryptic *Maxomys* species that differ in the morphology of the X chromosome. Further sampling and karyotyping will be required to address this point.

Berylmys berdmorei (Anderson, 1879) and *B. bowersi* (Blyth, 1851)

A single *B. berdmorei* female, as well as one male and one female of *B. bowersi* were karyotyped. All three specimens had $2n=40$. The karyotype comprised two large (pairs 1 and 2 in Fig. 4 and 5), four medium-size (pairs 5, 6, 9 and 11) and one small submetacentric pairs (pair 18), one large (pair 3), five medium-size (pairs 12-16) and one small metacentric pair (pair 17), and three medium-size (pairs 4, 7, 8 and 10) and one short acrocentric (pair 19) pairs of chromosomes (NFa=64; see Fig. 4 and 5). The X is a medium-size acrocentric chromosome and the *B. bowersi* Y is a small acrocentric element. Our results are in good agreement with previous reports (reviewed in Marshall and Newcomb, 1983) that suggested $2n=40$ and NFa=64 for both species. An additional female *B. berdmorei* specimen was found to have $2n=41$ due to the presence of an extra C-negative metacentric element that did not match with any of the other elements of the karyotype using G-banding comparison (Fig. 4) suggesting that this may be a B-chromosome. To our knowledge, this is the first report of B-chromosomes in this genus.

Our results show some differences to those of Yong (1968) and Yosida (1973) who identified six pairs of acrocentric, eight pairs of metacentric and five pairs of subtelocentric autosomes; they also show a medium sized acrocentric X chromosome and a small acrocentric Y in specimens of *B. bowersi*. This mirrors other conflicting reports regarding the *B. berdmorei* karyotype. Marshall (1977) described six telocentric, six subtelocentric and seven metacentric pairs of autosomes in *B. bowersi* from Thailand, whereas Yong (1968) recorded eight pairs of metacentrics, nine pairs of subtelocentrics, two pairs of acrocentric autosomes and a medium sized subtelocentric X in specimens from Malaysia. Conversely, Markvong *et al.* (1973) and Duncan *et al.* (1970) identified five pairs of subtelocentrics, eight pairs of metacentrics and six pairs of acrocentrics in this species in Thailand and Vietnam. The X and Y chromosomes of *B. bowersi* described by Yong (1968) and Yosida (1973) are comparable to our own. Conversely the *B. berdmorei* X has previously been reported as subtelocentric (Yong, 1968), while our specimens clearly possessed an acrocentric X, in agreement with both Duncan *et al.* (1970) and Markvong *et al.* (1973). Furthermore, we were unable to retrieve the two species-specific differences that were proposed to differentiate the karyotypes, i.e. a subtelocentric vs. acrocentric pair 6, and an acrocentric vs. subtelocentric pair 11 in *B. berdmorei* and *B. bowersi*, respectively (see Marshall, 1977). Given that our C-banding data show the presence of several C-positive short arms (Fig. 4 and 5) which may be responsible for variations in chromosome morphology (i.e., the interpretation of acrocentric vs. subtelocentric), it seems prudent to conclude that the two *Berylmys* species cannot be distinguished cytogenetically with any confidence.

Bandicota indica (Bechtein, 1800) and *B. savilei* Thomas, 1916

Eight bandicoot-rats, including two *B. indica* (one male, one female) and six *B. savilei* (four males, two females) specimens were karyotyped.

The *B. indica* specimens were characterized by $2n=44$ and 45 , respectively (data not shown). Unfortunately, well resolved G- and C-banded chromosomes could not be produced due to the poor quality of our preparations. That said, the $2n=45$ male clearly possessed 26 bi-armed chromosomes ($NF=71$) including a moderately large submetacentric X. The rest of the karyotype was composed of acrocentric elements, most probably including the Y (which would result in $NFa=68$) although its unambiguous identification was not possible from our preparations. The $2n=44$ female was characterized by the same karyotypic morphology, except that it possessed one more acrocentric but one less bi-armed chromosome (data not shown). Although the difference between the $2n=44$ and $2n=45$ karyotypes may suggest a Robertsonian polymorphism in *B. indica*, based on our data we are unable to reach a definitive conclusion. Consequently, other explanations (e.g., X-autosome translocations, B chromosomes) cannot be excluded at this stage. For example, *B. indica* has been previously reported to contain 1-3 B chromosomes (Gadi *et al.*, 1982; Gadi & Sharma, 1983). Importantly, however, our results are slightly different to those previously published for this species collected from Thailand ($2n=46$; Markvong *et al.*, 1973; Marshall, 1977), but are consistent with data on specimens from India ($2n=42$ for *B. indica indica*, and $2n=44$ for *B. indica nemorivaga*; Ranjini, 1967; Avirachan *et al.*, 1971; Gadi and Raman, 1977; Gadi & Sharma, 1983). When taken together, the variation in $2n$ suggests a more complex systematic situation than currently thought within *B. indica*, and/or that there is a chromosomal polymorphism that remains to be described.

Three males and two females of *B. savilei* were characterised by $2n=43$ (Fig. 6). The diploid number of an additional male specimen was determined to be $2n=45$. Unfortunately, as for *B. indica*, our preparations were of very poor quality thus greatly limiting the description of these karyotypes and our interpretation of their differences. The fundamental number could not be determined confidently from these five specimens. Nevertheless, we were able to establish a cell line for one female allowing for a more detailed investigation of the *B. savilei* karyotype. This specimen had $2n=43$ comprising 23 meta- and submetacentric elements (NF=64; Fig. 6). Among the bi-armed chromosomes two correspond to the large metacentric X pair (as confirmed by Zoo-FISH using a *Rattus norvegicus* X paint; Badenhorst *et al.*, unpubl.), and one was a B chromosome (Fig. 6a). C-banding showed that the X long arms as well as several acrocentric and metacentric centromeric regions were C-positive (Fig. 6b). More surprisingly, the B-chromosome did not appear strongly stained following C-band (Fig. 6b). We are not aware of previous reports of B-chromosomes in *B. savilei*.

The karyotype described above differs from previous reports for specimens of this species collected in Thailand ($2n=44$, NFa=58; Markvong *et al.*, 1973). Markvong *et al.*, (1973) described a karyotype consisting of six pairs of small metacentrics and two pairs of subtelocentrics and thirteen pairs of acrocentrics. Also, the largest autosomal pair was clearly submetacentric. Furthermore, the X of *B. savilei* was previously identified as a medium-sized acrocentric which differentiates it from other lesser bandicoot rats (Markvong *et al.*, 1973; Gadi & Sharma, 1983). In absence of comparable data, we are unable to explain such differences. However, in our specimen the X was identified unambiguously by Zoo-FISH analysis (Badenhorst *et al.*, unpubl.) as a large submetacentric chromosome. Therefore, males of *B. savilei* should have a $2n=43+1B$ karyotype (i.e., XY1Y2 males). Clearly, this requires confirmation by further cytogenetic investigations.

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368 *Hapalomys delacouri* Thomas, 1927

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370 A fibroblast cell culture was established from a male *H. delacouri* specimen caught in the
 371 vicinity of Loei, and our data are the first for this species from Thailand. Its karyotype was
 372 characterized by $2n=48$ chromosomes and an $NFa=92$ (Fig. 7). All the autosomes were bi-
 373 armed (metacentric or submetacentric). The metacentric X and the acrocentric Y were easily
 374 recognizable since they were respectively the largest and the smallest elements in the
 375 karyotype. Our findings differ significantly from published data on *H. longicaudatus* (Yong *et*
 376 *al.*, 1982) in that these authors describe specimens from Malaysia as having $2n=50$, with the
 377 X and Y chromosomes having similar morphology; all the autosomes were reported as
 378 acrocentric. This suggests that cytotaxonomy may be a valuable diagnostic tool within the
 379 genus. Our results constitute the first karyotypic data for this very poorly documented species.
 380 Interestingly, two classes of C-positive material were identified on staining intensity
 381 suggesting a different repeat composition for this heterochromatic material (Fig. 7b). The
 382 genome appears to be particularly rich in heterochromatin, with some chromosome pairs
 383 displaying entirely heterochromatic long arms.

384

385 *Chiropodomys gliroides* (Blyth, 1856)

386

387 A fibroblast cell culture was established from a male *C. gliroides* specimen that possessed an
 388 entirely acrocentric chromosomal complement ($2n=42$, $NF=42$), including the X and Y
 389 chromosomes ($NFa=40$). Unfortunately, the preparations were of poor quality and
 390 construction of a G-banded karyotype was not possible. Nevertheless, strongly positive C-
 391 bands could be clearly observed in all pericentromeric regions, whereas the Y chromosome

was entirely heterochromatic, although less heteropycnotic than the pericentromeric material of the autosomes (Fig. 8). These results are in partial agreement with previous data concerning this very poorly documented species. Yong (1973, 1983) reported that eight Malayan specimens were characterized by $2n=42$ with most chromosomes being acrocentric in morphology with C-positive pericentromeric regions (Yong, 1983). Additionally there was one metacentric chromosome pair, a submetacentric chromosome pair, and the sex chromosomes were bi-armed. These bi-armed elements were all clearly absent in our single specimen of this species (Fig. 8). At this point, we cannot distinguish whether the differences are intraspecific (i.e. represent polymorphisms), or that they underpin the recognition of apparently cryptic species within *C. gliroides*.

Mus caroli (Bonhote, 1902), *M. cervicolor* Hodgson, 1845 and *M. cookii* Ryley, 1914

Seven male and seven female mice, trapped close to or within pineapple fields in Loei, were characterized by an identical $2n=40$ acrocentric karyotype ($NFa=38$). The karyotypes of the Thailand species *M. musculus castaneus*, *M. caroli*, *M. cervicolor*, *M. cookii*, *M. shortridgei* and *M. fragilicauda* (see Auffray *et al.*, 2003, and references therein) all have the same invariant karyotype (Auffray *et al.*, 2003) indistinguishable from that evidenced by our specimens. Molecular typing using rDNA16S and rDNA28S nucleotide sequences of the 14 specimens analysed by us allowed the identification of three different species in our sample, *M. caroli*, *M. cervicolor* and *M. cookii* (A. Orth, pers. comm.). Several of these animals were caught in the same trap lines, often only meters apart, thus representing another report of true syntopy as has been similarly suggested for *M. caroli*, *M. cervicolor* and *M. fragilicauda* in Thailand (Auffray *et al.*, 2003).

In addition, two female specimens, identified as *M. cookii* on nucleotide sequences (A. Orth, pers. comm.), had $2n=41$ chromosomes (Fig. 8). The two karyotypes each comprised 40 acrocentric chromosomes, in addition to one unpaired medium-size metacentric and one unpaired acrocentric element respectively. These two unpaired chromosomes are G-negative (not shown) and C-positive (Fig. 8b) and are considered by us to be B-chromosomes. To our knowledge, this is the first report of B-chromosomes in the genus *Mus*. Although several hypotheses have been proposed to explain some aspects of the biology of these extra, non-Mendelian chromosomes (reviewed in Camacho *et al.*, 2000), little is known about their origin and their impact on the genomes, especially in mammals. Keeping in mind the importance of the mouse as a biological model, this observation clearly merits further investigation.

Conclusion: chromosomal evolution in Thai rodents and epidemiological implications

Despite a central role as reservoirs and/or vectors of several human diseases, reliable systematic data necessary to underpin accurate species-specific geographical, ecological and genetic information on Asian rodents are limited, and many taxonomic associations are unclear. Cytotaxonomy has been of great help in similar epidemiological investigations on other continents i.e., Africa), frequently providing unambiguous identification of species including those that are morphologically cryptic (e.g., Duplantier *et al.*, 1990; Volobouev *et al.*, 2002; Dobigny *et al.*, 2003; Granjon & Dobigny, 2003; Rambau *et al.*, 2003; Veyrunes *et al.*, 2004). However, apart from *Hapalomys*, the preliminary results presented here suggest that this does not hold for the Asian species surveyed in our study. Although most genera can routinely be discriminated on karyotype (as they often can on morphology), few species-specific chromosomal characters could be identified within genera investigated herein. For

instance, although *L. neilli* and *L. edwardsi* could be delimited cytogenetically, the two *Berylmys*, three *Mus*, three *Rattus* species analysed by us are indistinguishable on karyotype. Although it remains to be confirmed, a similar situation may exist within *Niviventer*, as well as between *L. edwardsi* and *L. sabanus*. Karyotypic criteria that have previously been claimed to be diagnostic appear in our analyses to simply represent ancestral polymorphisms that are still undergoing idiosyncratic lineage sorting (i.e., hemiplasic characters *sensu* Avise & Robinson, 2008; Robinson *et al.*, 2008), thus impacting on the usefulness of these as diagnostic markers in species identification. Our findings suggest that new diagnostic features should be sought as a matter of urgency for use in these epidemiologically important rodent groups. DNA-based methods such as primer-specific analysis that have been used successfully in Africa (e.g., Lecompte *et al.*, 2005) and Europe (e.g., Michaux *et al.*, 2001) are compelling candidates that should be prioritised for testing in Asian murids.

Acknowledgements

This study is part of the « Biodiversité Comparée des Roboviroses à Hantavirus en Europe et en Asie du sud-est » programme (ANR Project n°00121-05, J.P. Hugot). Daleen Badenhorst research is supported by a NRF bursary and a bilateral France/South Africa Protea grant to GD and TJR. We are grateful to Annie Orth and Josette Catalan for their help in the lab, Jo and Kaï (drivers), Kittiponk Chaisiri, Khomson Satchasataporn, the staff and students from the Faculty of Veterinary Technology, Kasetsart University, as well as the villagers in Loei and Phrae for their help in preparing and assisting the fieldwork. Cell culture experiments were performed using facilities provided by the “Evolutionary consequences of inversion polymorphism in Mammals” programme (ANR Project n°ANR05-JC05-48631, G. Dobigny) and the Evolutionary Genomics Group, University of Stellenbosch.

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Table and Figure Legends

Table 1: Specimens investigated in the present study. Those that were diagnosed using DNA sequencing are noted as “+” in the Id. DNA column. Individual numbers refer to the CBGP Asian rodent collection (resp. Y. Chaval, Montpellier, France). Sex, origin, diploid (2n) and autosomal fundamental (NFa) numbers, type of biological material (bm: bone marrow; cc: cell culture), sex chromosomes morphology (X/Y) as well as number of B-chromosomes (Bs) are provided for each animal.

Table 2: Chromosomal polymorphism observed for pairs 1, 9 and 13 (*sensu* Yosida and colleagues; see text for details) in the *Rattus* specimens investigated here. M and A correspond to meta-/submetacentric and acrocentric morphs, respectively. Like for Table 1, individual numbers refer to the CBGP Asian rodent collection (resp. Y. Chaval, Montpellier, France).

Fig. 1: G-banded karyotype (a) and C-banded metaphase (b) of a male *Leopoldamys edwardsi* (n°R5239). Arrows indicate C-positive intercalary bands and minute Y.

Fig. 2: G-banded karyotype (a) and C-banded metaphase (b) of a female *Niviventer fulvescens* (n°R4519).

Fig. 3: G-banded karyotype (a) and C-banded metaphase (b) of a male *Maxomys surifer* (n°R4404). The arrow indicates the C-positive Y chromosome.

Fig. 4: G-banded karyotype (a) and C-banded metaphase (b) of a female *Berylmys berdmorei* (n°R4406).

Fig. 5: G-banded karyotype (a) and C-banded metaphase (b) of a male *Berylmys bowersi* (n°R4400). The arrow indicates the C-positive Y chromosome.

Fig. 6: G-banded karyotype (a) and C-banded metaphase (b) of a female *Bandicota savilei* (n°R4408). Arrows indicate the X chromosomes.

Fig. 7: G-banded karyotype (a) and C-banded metaphase (b) of a male *Hapalomys delacouri* (n°R5237). The arrow indicates the C-negative Y chromosome.

Fig. 8: C-banded metaphase of a male *Chiropodomys gliroides* (n°R5238).

Fig. 9: Conventionally stained (a) and C-banded (b) metaphases of one female *Mus cooki* (n°R4105) showing the strongly C-positive and metacentric B-chromosome (arrows).

646 Table 1

647

Species	Id. DNA	Individuals			material	Cytogenetics			
		individual	sex	origin		2N	X/Y	NFa	Bs
<i>Menetes berdmorei</i>	-	R4002	F	Kalasin	bm	38		72	
<i>Rattus losea</i>	+	R4203	F	Phrae	bm	42	A	58	
	+	R4229	M	Loei	bm	42	A/A	58	
	+	R4230	M	Loei	bm	42	A/A	57	
	+	R4260	F	Loei	bm	42	A	58	
	+	R4724	F	Loei	cc	42	A	56	
<i>Rattus tanezumi</i>	+	R4113	M	Loei	bm	42	A/A	54	
	+	R4481	F	Phrae	bm	42	A	55	
	+	R4003	F	Kalasin	bm	42	A	57	
	-	R4008	M	Kalasin	bm	42	A/A	55	
	+	R4016	F	Phrae	bm	42	A	52	
	-	R4096	F	Loei	bm	42	A	52	
	-	R4145	F	Phrae	bm	42	A	54	
	+	R4182	F	Phrae	bm	42	A	56	
	+	R4436	M	Phrae	bm	43	A/A	59 ?	1
	-	R4447	M	Phrae	bm	42	A/A	55	
	-	R4450	M	Phrae	bm	42	A/A	56	
	-	R4542	F	Loei	bm	42	A	56	
	+	R4075	M	Loei	bm	42	A/A	57	
<i>Rattus exulans</i>	-	R4033	F	Phrae	bm	42	A	58	
	-	R4035	F	Phrae	bm	42	A	59	
	-	R4100	F	Loei	bm	42	A	59	
	+	R4103	M	Loei	bm	42	A/A	58	
	-	R4104	M	Loei	bm	42	?	?	
	-	R4112	M	Loei	bm	42	A/A	54	
	+	R4140	F	Phrae	bm	42	A	?	
	-	R4216	M	Loei	bm	42	A/A	58	
<i>Bandicota indica</i>	+	R4000	M	Kalasin	bm	45	??	?	
	+	R4265	F	Loei	bm	44	?	?	
<i>Bandicota savilei</i>	+	R4141	M	Phrae	bm	43	??	?	
	+	R4142	M	Phrae	bm	43	??	?	
	+	R4143	M	Phrae	bm	45	??	?	
	+	R4017	F	Phrae	bm	43	?	?	
	+	R4408	F	Loei	cc	43	SM	60	1
	+	R4021	M	Phrae	bm	43	??	?	
<i>Leopoldamys edwardsi</i>	+	R5239	M	Loei	cc	42	A/A	54	
	+	R5240	M	Loei	cc	42	A/A	54	
	+	R4070	M	Loei	bm	42	A/A	54	
	+	R4098	M	Loei	bm	42	A/A	54	

	+	R4222	F	Loei	bm	42	A	54	
<i>Leopoldamys neilli</i>	+	R4477	F	Phrae	bm	44	A?	50	
<i>Maxomys surifer</i>	+	R4099	M	Loei	bm	52	A/A	64	
	+	R4107	M	Loei	bm	52	A/A	64	
	+	R4108	M	Loei	bm	52	A/A	64	
	+	R4223	M	Loei	bm	52	A/A	64	
	+	R4259	F	Loei	bm	52	A	64	
	+	R4404	M	Loei	cc	52	A/A	64	
<i>Niviventer fulvescens</i>	+	R4071	M	Loei	bm	46	A/A	52	
	+	R4409	M	Loei	cc	44 (rearr.)			
	+	R4519	F	Loei	bm	46	A	52	
<i>Berylmys berdmorei</i>	+	R4266	F	Loei	bm	40	A	62	
	+	R4406	F	Loei	cc	41	A	64	1
<i>Berylmys bowersi</i>	+	R4102	F	Loei	bm	40	A	62	
	+	R4400	M	Loei	cc	40	A/A	62	
<i>Hapalomys delacouri</i>	+	R5237	M	Loei	cc	48	M/A	92	
<i>Chiropodomys gliroides</i>	+	R5238	M	Loei	cc	42	A/A	40	
<i>Mus cooki</i>	+	R4074	M	Loei	bm	40	A/A	38	
	+	R4105	F	Loei	bm	41	A	40	1
	+	R4215	M	Loei	bm	40	A/A	38	
	+	R4097	M	Loei	bm	40	A/A	38	
	+	R4101	F	Loei	bm	40	A	38	
	+	R4106	M	Loei	bm	40	A/A	38	
	+	R4110	M	Loei	bm	40	A/A	38	
	+	R4111	F	Loei	bm	40	A	38	
	+	R4146	F	Phrae	bm	40	A	38	
	+	R4264	F	Loei	bm	40	A	38	
	+	R4147	F	Phrae	bm	41	A	39	1
<i>Mus cervicolor</i>	+	R4109	M	Loei	bm	40	A/A	38	
	+	R4183	M	Phrae	bm	40	A/A	38	
<i>Mus caroli</i>	+	R4240	F	Loei	bm	40	A	38	

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650

651 Table 2

652

Species	Individuals	Polymorphism in <i>Rattus</i> species		
		pair 1	pair 9	pair 13
<i>Rattus losea</i>	R4203	MM	MM	AA
	R4229	MM	MM	AA
	R4230	MM	MM	AA
	R4260	MM	MM	AA
	R4724	MM	AA	AA
<i>Rattus tanezumi</i>	R4113	AA	AA	AA
	R4481	AA	AM	AA
	R4003	AM	AM	AM
	R4008	AA	AM	AA
	R4016	AA	AA	AA
	R4096	AA	AA	AA
	R4145	AA	AA	AA
	R4182	AA	MM	AA
	R4436	AA	?	?
	R4447	AM	AA	AA
	R4450	AM	AM	AA
	R4542	AA	MM	AA
	R4075	AM	MM	AA
<i>Rattus exulans</i>	R4033	MM	MM	AA
	R4035	MM	MM	AA
	R4100	MM	MM	AM
	R4103	MM	MM	AA
	R4104	?	?	?
	R4112	MM	AA	AA
	R4140	?	?	?
	R4216	MM	AA	MM

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

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Fig. 6

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Fig. 7

Fig. 8

688 Fig. 9

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